Denitrification and nitrogen fixation in floodplain successional soils along the Tanana River, interior Alaska

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Forest floors and mineral soils from early (open willow), middle (poplar–alder), and late (white spruce) floodplain primary successional stages were examined for nitrogen fixation and denitrification. The acetylene-reduction and acetylene-inhibition techniques were used separately and in combination to measure nitrogenase and denitrification activities, both in laboratory and field studies. In situ N_2O production was undetectable at all sites and during all sampling periods. Denitrifying activity measured in the field with acetylene amendments was low to undetectable, except after a brief flood in the open willow stand when N_2O production ranged from undetectable to 34 ng $N \cdot cm^{-2} \cdot h^{-1}$ within the newly deposited alluvium – old mineral soil interface. Intact core assays also had low to undetectable denitrification activities; the highest activities (259 ng $N \cdot g^{-1} \cdot h^{-1}$) were measured in the poplar–alder forest floor in the fall. Laboratory studies showed that potential denitrification enzyme activity (DEA) was also greatest in the poplar–alder forest floor (4332 ng $N \cdot g^{-1} \cdot h^{-1}$), once again occurring in the fall. In early and midsuccessional stages, the interactive effects of temperature, carbon, and NO_3^- limited denitrification, yet even with the addition of the limiting amendments, low to undetectable DEA was observed in mineral soils. The later white spruce successional stage also had low to undetectable DEA, increasing only with the addition of the full DEA media and independent of temperature changes. Nonsymbiotic nitrogenase activities were highly variable, ranging from undetectable to 30 ng $N \cdot cm^{-2} \cdot h^{-1}$. Highest activities were seen in the open willow, newly deposited alluvium – old mineral soil interface immediately after a flood and approximately 1 month after the flood on the newly deposited silt surface. Only the white spruce forest floor had measurable nonsymbiotic nitrogenase activity at all sampling times. Alder root nodule nitrogenase activity showed no significant differences between sampling periods. The estimated annual nitrogen fixation rate of 164 kg $N \cdot ha^{-1}$ for alder root nodules is a substantial N contribution to the alder stand and to the floodplain ecosystem in general.

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Les couvertures mortes et les sols minéraux des stades de succession juvénile (saulaie ouverte), de mi-succession (peuplier–aulne) et de succession avancée (épinette blanche) d'un plaine de débordement ont été examinées pour la fixation d'azote et la dénitrification. Les techniques de réduction d'acétylène et d'inhibition d'acétylène ont été utilisées séparément et en combinaison pour mesurer les activités de la nitrogénase et de la dénitrification, à la fois dans les études menées au laboratoire et au champ. La production *in situ* de N_2O était non détectable dans toutes les stations et pour toutes les périodes échantillonnées. L'activité dénitrifiante mesurée au champ avec les amendements d'acétylène variait de faible à non détectable, excepté après une brève période d'inondation dans la saulaie ouverte, quand la production de N_2O a varié de non détectable à 34 ng $N \cdot cm^{-2} \cdot h^{-1}$ à l'interface des alluvions nouvellement déposées et du vieux sol minéral. Les essais sur des prélèvements non perturbés ont aussi montré des activités de dénitrification variant de faible à non détectable, les activités les plus élevées étaient mesurées dans la couverture morte de peuplier–aulne, 259 ng $N \cdot g^{-1} \cdot h^{-1}$, à l'automne. Les études en laboratoire ont montré que l'activité potentielle de l'enzyme de dénitrification (DEA) était aussi la plus forte dans la couverture morte de peuplier–aulne, 4332 ng $N \cdot g^{-1} \cdot h^{-1}$, encore une fois survenant à l'automne. Dans les stades juvénile et de mi-succession, les effets interactifs de la température, du carbone et de NO_3^- ont limité la dénitrification, même avec l'ajout des amendements limitants, une DEA de faible à non détectable a été observée dans les sols minéraux. Le stade successionnel plus avancé d'épinettte blanche avait aussi une DEA de faible à non détectable, augmentant seulement avec l'addition d'un milieu complet de DEA et indépendant des changements de température. Les activités non symbiotiques de la nitrogénase étaient fortement variables, de non détectable à 30 ng $N \cdot cm^{-2} \cdot h^{-1}$. Les activités les plus élevées ont été observées dans la saulaie ouverte, à l'interface des alluvions nouvellement déposées et du vieux sol minéral, immédiatement après une inondation et approximativement 1 mois après l'inondation sur les surfaces de nouveaux dépôts de limons. Seulement la couverture morte de la forêt d'épinette blanche avait une activité mesurable de la nitrogénase non symbiotique pour toutes les périodes d'échantillonnage. L'activité nitrogénase des nodules d'aulne n'a pas montré de différence significative entre les périodes d'échantillonnage. Le taux annuel de fixation d'azote, estimé à 164 kg $N \cdot ha^{-1}$ pour les nodules d'aulne, est une contribution substantielle de N au peuplement d'aulne et à l'écosystème de la plaine de débordement en général.

[Traduit par la rédaction]

Introduction

Generally, it is believed that symbiotic N fixation is the major mechanism of soil N buildup in early stages of primary succession (Crocker and Major 1955; Connell and Slatyer 1977; Reiners 1981). In older forest ecosystems where nitrogen cycling is tightly coupled, low rates of nonsymbiotic nitrogen fixation are thought to compensate for N losses due to denitrification or leaching (Nohrstedt 1984; Alexander and Billington 1986; Grant and Binkley 1987). Low oxygen tensions along with available nitrate and organic matter substrates are necessary requirements for denitrification to occur (Tiedje et al. 1989); thus in early stages of primary succession, dentrification may not be significant. In later stages, with organic matter accumulation, increases in total N, and

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increases in overall soil respiration, dentrification activity would be expected to increase.

A single theory cannot explain the wide variations of N-cycling in different ecosystems (Vitousek et al. 1989). However, the generalization that total N and available N increase with time during primary succession does seem to hold despite differences in types of primary succession. These differences can be due to (*i*) the presence of organic matter and N immediately after disturbance, (*ii*) whether dominant plants have a symbiotic N-fixing association in early succession, and (*iii*) changes in the proportion of N mineralized per unit of soil N, with soil age (Vitousek et al. 1989). Stages within a successional sere can also differ substantially in relation to soil fertility and soil physical attributes (Van Cleve et al. 1993, this issue). Moreover, environmental factors can also vary within a stage on a microsite scale, in part explaining the high degree of spatial and temporal variability that is evident with N processes (Schimel et al. 1988).

Nitrogen cycling has in large part been studied by observing single processes over different spatial and temporal scales. Individually, both N fixation and denitrification have been extensively researched, but most of this research has been devoted to agriculture and much remains to be understood of the controls and functions in natural environments. The object of this study was to examine N fixation and denitrification within primary-successional floodplain soils of interior Alaska.

Study area

The study area is adjacent to the Bonanza Creek Experimental Forest, approximately 20 km southwest of Fairbanks, Alaska. Three of the 12 described successional stages were chosen to represent early, middle, and late primary succession (Viereck et al. 1993, this issue). These are as follows: III-A-C, a 5-year-old open willow (*Salix* spp.) stand; V-A-C, a 27-year-old poplar–alder stand (open balsam poplar (*Populus balsamifera* L.) with a dense thinleaf alder (*Alnus tenuifolia* Nutt.) understory); and VIII-A-C, a 165-year-old white spruce (*Picea glauca* (Moench) Voss) stand. Detailed descriptions of the soils and vegetation of each of the three stages are found in Viereck et al. (1993, this issue). Ages of each of the stands are from approximate date of sandbar formation. Each designated plot was an area of 50 × 50 m. Soils were sampled along randomly selected transects that ran perpendicular to the river and spanned the width of each plot. Both forest floors and mineral soils of the poplar–alder and white spruce stands were sampled and tested independently. The open willow stand did not have a forest floor, thus only mineral soil was sampled and tested. Soil samples were collected equidistantly along transects with a 15 cm diameter soil corer to approximately a 12–15 cm depth.

Materials and methods

In situ nitrogen fixation and denitrification

In early July, 10 replicate open-ended amyl–butyl–styrene (ABS) tubes (12 cm diameter and 15 cm high) were placed at uniform intervals, to depths of 8–10 cm below the surface of the forest floor, along a transect at each site. A second transect was placed in the open willow stand immediately after water from a flood (July 22, 1986) receded. These tubes were used to sample the 8–15 cm of newly deposited alluvium, and the earlier placed tubes were used as sampling chambers for the interface between the new alluvium and old surface soil. Both in situ denitrification, using the acetylene-inhibition technique (Tiedje 1982), and in situ nitrogen fixation, using the acetylene-reduction technique (Stewart et al. 1967), were measured with these tubes. Vacuum stopcock grease was applied to the exposed rim of each tube and capped with a Plexiglas lid, fitted with a rubber septum to allow gas sampling of the headspace. Acetylene, generated by the addition of CaC_2 to distilled water, was added to sealed ABS tubes at a concentration of 10–20% (v/v). Gas traps constructed of closed-ended Plexiglas cylinders (8 cm diameter by 2 cm high with twelve 0.05 cm diameter holes in the bottom) were placed at 0–5, 5–10, 20–25, and 50–55 cm depth from the surface of the soil. Each gas trap had a 0.05-cm plastic capillary tube that extended to the surface of the soil. The end of the tubing had a rubber septum used for soil atmosphere sampling. These gas traps served as controls (no acetylene additions) for both ethylene and nitrous oxide production. Incubations for acetylene-amended ABS tubes were from 3 to 5 h. Replicate 5-mL gas samples were collected from both the ABS tubes and gas traps and stored in evacuated glass tubes for N_2O, C_2H_2, and C_2H_4 analyses. The ABS tubes and gas traps were sampled every 2 weeks from July until September.

Because of the extremely low to undetectable amounts of N_2O measured within the ABS tubes, an experiment was designed to test the capability of the sealed ABS tubes to accumulate N_2O. In addition, this was used to estimate a field denitrification enzyme activity (DEA) (Smith and Tiedje 1979; Tiedje et al. 1989). Four replicate ABS tubes were used as controls (no additions other than acetylene), while another four tubes were saturated with a medium containing 1.44 $g \cdot L^{-1}$ KNO_3, 1.00 $g \cdot L^{-1}$ dextrose, and 0.25 $g \cdot L^{-1}$ chloramphenicol (complete DEA medium) (Smith and Tiedje 1979). Chloramphenicol, a protein-synthesis inhibitor, was added so that only preexisting denitrification enzymes were measured. Immediately after the addition of the complete DEA medium, all eight ABS tubes were sealed and sampled at intervals of 1 h, for 5 h. All gas samples were stored in glass evacuated tubes for N_2O analyses. Concentrations of N_2O were measured by electron capture (^3H) gas chromatography using a 1.8-m Poropak Q column at 65°C and a detector temperature of 153°C. An argon–methane (95%:5%) mixture was used as the carrier gas. The limit of detection was 0.6 ng $N_2O\text{-}N \cdot cm^{-2} \cdot h^{-1}$.

Intact core and denitrifying enzyme activity

Twenty cores (5 cm in diameter including the forest floor and approximately 2–5 cm of mineral soil) collected along transects at each site were brought to the laboratory for intact core and DEA (Smith and Tiedje 1979; Tiedje et al. 1989) measurements during May, June, July, and September. Ten cores were kept intact, with the exception of separating the forest floor layer from the mineral soil of the alder and white spruce, and were placed in half-pint canning jars with lids equipped with rubber septa. The other 10 cores, used for enzyme assays, were made into two composite samples: forest floor and mineral soil, with each fraction separately mixed. Quadruplicate subsamples, 25–50 g fresh weight, of the mixed composites were placed in similar half-pint canning jars. These soil samples were then made into a slurry by the addition of the complete DEA medium equal in mass to that of the forest floor and mineral soil samples. Samples were then made anaerobic by flushing with N_2 five times. DEA assays and one-half of the intact core samples were amended with a headspace addition of 10% (v/v) acetylene. At the end of the incubation period, 2 h continual shaking for the DEA assays and 24 h for the intact cores, a 5-mL gas sample of each jar was taken and stored in a glass evacuated tube for N_2O analysis.

Another set of quadruplicate mixed composite subsamples, from each of the three sites, was taken to evaluate the effects of temperature, moisture, nitrate, dextrose, and anaerobiosis on DEA. These factors were tested at four temperatures (5, 10, 20, and 30°C) in conjunction with six treatments: A, control (no additions); B, distilled water added to water holding capacity; C, anaerobic atmosphere (no water added); D, nitrate addition; E, dextrose addition; and F, an all-inclusive treatment that consisted of an anaerobic atmosphere and the addition of the complete DEA medium. Individual treatments were of the same amount and concentration as that of the denitrifying enzyme media addition in a 1:1 solution to sample ratio. Water holding capacity had been previously determined by saturating soil samples with distilled water for 24 h with subsequent drainage for 24 h. Incubations were for 6 h, at which time a 5-mL gas sample of each jar was taken and stored.

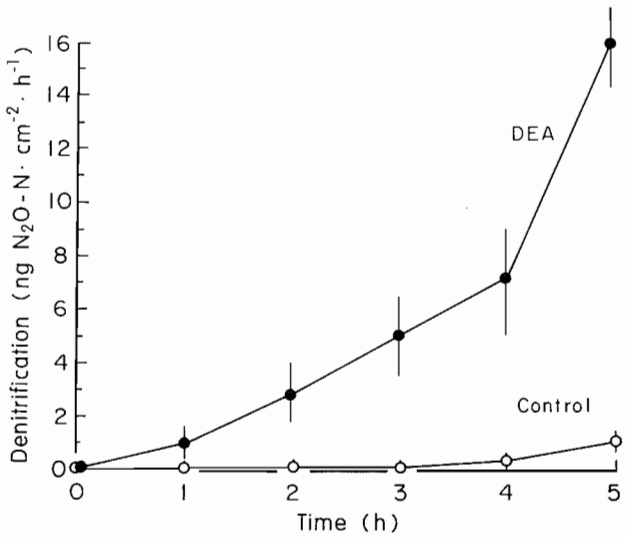

FIG. 1. Poplar–alder stand denitrification activity within the in situ ABS core tubes with and without the addition of the complete DEA medium. Data are presented as means ± 1 SE (n = 4).

Nitrogen fixation assays

Alder root nodule nitrogenase activity, in the poplar–alder stand, was measured using excised nodules. Alder root nodules were collected within 10 subplots (25 × 25 cm) located along selected transects, at time intervals of approximately 2 weeks beginning in July and continuing until September. Usually several root nodule clumps were found within each subplot, and all clumps found within each subplot were collected and treated as one sample. Immediately after excising, the nodules were placed in a half-pint canning jar, which was sealed with a lid equipped with a rubber septum, and a 10% (v/v) headspace volume of acetylene was added. Incubations were carried out in the field, in a shaded area, for 2 h. At the end of the incubation period a gas sample of the headspace of each jar was taken and stored in a glass evacuated tube until ethylene analysis could be completed. Root nodules hand sorted from 30 cores (10 cores, 15 cm diameter to approximately 12–15 cm soil depth taken along three transects) and nodules collected at each subplot (at each sampling period) were used to determine nodule weight per area. All nodules were dried at 65°C for 48 h.

The effects of temperature and moisture were also tested on the nonsymbiotic nitrogenase activity of forest floors and mineral soils at each site. Forest floor material in the white spruce stand included living moss. Four temperatures (5, 10, 20, and 30°C) and two moisture regimes (field moisture content and soil water holding capacity) were used. For each temperature and moisture combination, six replicate intact cores (5 cm in diameter and 2–5 cm in length) were placed in individual half-pint canning jars and sealed with lids equipped with rubber septa. With these cores, the forest floor and mineral soil of each were carefully separated and treated independently. Cores from the poplar–alder stand were checked for alder nodules before assays began. Samples were incubated for 24 h in the presence (10% (v/v) addition) and absence of acetylene. At the end of the incubation period, gas samples were collected from each jar and stored in glass evacuated tubes for C_2H_4 analyses. A gas chromatograph equipped with a H_2 flame-ionization detector was used for the detection of C_2H_4. Nitrogen was used as the carrier gas in conjunction with a 1.8-m Poropak R (100–120 mesh) column with a detector temperature of 60°C. The detection limit for C_2H_4 was 0.01 ng $C_2H_4 \cdot cm^{-2} \cdot h^{-1}$.

For each jar assay, incubated material was dried (forest floor at 65°C for 48 h and mineral soil at 105°C for 48 h) and weighed to determine dry weights and the rate of reaction per unit sample weight. Total airspace volume in each of the sampling jars was calculated by the addition of water.

TABLE 1. Laboratory intact core denitrification (acetylene amended) in both mineral soils (MS) and forest floors (FF) of the open willow, poplar–alder, and white spruce stands

Soil	Rate of denitrification (ng N_2O-N · $g^{-1} \cdot d^{-1}$)			
	May	June	July	September
Open willow MS	—	0.4±0.5*b*	0.7±0.5*c*	—
Poplar–alder MS	—	—	12±1*a*	—
Poplar–alder FF	—	13±2*a*	18±8*a*	259±122*a*
White spruce MS	—	1±1*b*	0.7±0.7*c*	—
White spruce FF	—	1±0.7*b*	4±2*b*	—

NOTE: Each value is a mean ± 1 SE (n = 4). Values in a column followed by the same letter are not significantly different ($P < 0.05$). —, Undetected.

Analyses of variance (Zar 1984) were performed utilizing the Statistical Analysis System (SAS Institute Inc. 1985). Tukey's multiple range test (Zar 1984; SAS Institute Inc. 1985) was used to detect significant differences in estimates of denitrification and nitrogen fixation.

Results

Although the field DEA experiment did show that core tubes placed in the ground were capable of retaining nitrous oxide over time (Fig. 1), denitrifying activity (measured with acetylene addition) was below the limit of detection at all sites except for two sampling periods in August, when both the poplar–alder stand and the open willow stand had measurable amounts. Denitrifying activity measured during these two sampling periods was low and close to our limits of detectability. On August 8 and 15, poplar–alder stand ABS tubes ranged from undetectable to 0.8 ng N_2O-N · $cm^{-2} \cdot h^{-1}$, whereas those in the open willow stand ranged from undetectable to 34.0 ng N_2O-N · $cm^{-2} \cdot h^{-1}$. This activity measured in the open willow stand was only found within the newly deposited alluvium – old mineral soil interface. There were no measurable amounts of soil atmosphere N_2O at any depth from gas traps, during any sampling time.

The results of laboratory intact core denitrification assays showed no measurable amounts of N_2O production in soil samples that were not amended with acetylene. Acetylene-amended intact cores of the mineral soils at each site and the white spruce forest floor had undetectable to low rates of denitrification (Table 1). The poplar–alder forest floor intact cores had the highest denitrifying activity in September 1986 (259 ng N · $g^{-1} \cdot d^{-1}$) but showed little or no activity during other sampling times. The DEA assays had rates that were substantially higher than those of the intact cores, with the greatest activity occurring in the poplar–alder forest floor during all four sampling dates (Table 2). Highest rates for any of the stages tested were observed in September. Of all the assays used to measure denitrifying activity, this DEA assay (2-h incubation that was continually shaken) was the only one to show denitrification in the white spruce forest floor. The white spruce forest floor DEA rate in September was higher than the rate for any of the mineral soils in September (Table 2). Yet when converted to an area rate, using a bulk density conversion factor, the DEA rate for the poplar–alder mineral soil was significantly higher than the rate for any other soil: poplar–alder mineral soil, 71 mg N_2O-N · $m^{-2} \cdot d^{-1}$, white spruce forest floor, 18 mg N_2O-N · $m^{-2} \cdot d^{-1}$; open willow mineral soil, 0.6 mg N_2O-N · $m^{-2} \cdot d^{-1}$; and white spruce mineral soil, 0.5 mg N_2O-N · $m^{-2} \cdot d^{-1}$.

TABLE 2. Laboratory DEA assays in both mineral soils (MS) and forest floors (FF) of the open willow, poplar–alder, and white spruce stands

Soil	Rate of denitrification (ng N_2O-N·g^{-1}·d^{-1})			
	May	June	July	September
Open willow MS	0.8±0.4*b*	0.7±0.1*b*	13±0.6*b*	1±0.2*b*
Poplar–alder MS	0.9±0.7*b*	0.2±0.2*b*	54±14*b*	70±10*b*
Poplar–alder FF	77±10*a*	28±47*a*	42±42*a*	4332±996*a*
White spruce MS	—	0.1±0.1*b*	10±1*b*	1±0.4*b*
White spruce FF	1±0.4*b*	4±1*b*	28±2*b*	217±18*b*

NOTE: Each value is a mean ± 1 SE ($n = 4$). Values in a column followed by the same letter are not significantly different ($P < 0.05$). —, Undetected.

When testing for the limiting factors of denitrification, the highest rates of denitrification were measured in poplar–alder forest floor samples. Regardless of substrate additions or temperature increases, undetectable to low rates of denitrification were observed in all mineral soils and in white spruce forest floor samples (Table 3). In both the open willow and poplar–alder mineral soil, once temperatures increased to 20 and 30°C, the addition of the complete DEA medium increased the denitrifying activity substantially. Yet, increases were also observed with just the nitrate amendment. In the later, white spruce successional stage, denitrifying activity did not increase with an increase in temperature; only with the complete DEA medium, or nitrate addition in the forest floor samples, was there any activity. Within the poplar–alder forest floor significant differences ($P < 0.05$) were observed in denitrifying activity with the addition of treatments and with an increase in temperature (Fig. 2). At 5 and 10°C, all treatments had similar low denitrifying activities except for the samples amended with the complete DEA assay, i.e., treatment F. As temperature increased to 20 and 30°C, DEA increased with all treatments except for the control.

Nonsymbiotic nitrogenase activity measured within the three successional stands varied significantly among the soils tested (Table 4). The results suggest some seasonality among the sampling times. The open willow stage, in both the newly deposited alluvium and the interface of the new alluvium – old mineral soil, had detectable rates of nitrogenase activity on some sampling dates. Highest rates within the freshly deposited alluvium were observed late in the summer, whereas the highest rates in the new–old alluvial interface were observed immediately after the flood. The white spruce stand had higher nitrogenase activities in late July and mid-August, whereas the poplar–alder stand exhibited the lowest activities throughout the sampling period. There were no observed responses of nonsymbiotic nitrogenase activity to the laboratory temperature and moisture experiments from any of the three successional stands. In general, the alder root nodule nitrogenase activity was at least 1000 times greater than any of the measured nonsymbiotic nitrogenase activity. There was no detectable seasonal variation, although measurements taken on the same day exhibited extremely high variability (Table 5).

Discussion

The gas trap analyses of soil atmospheres indicated in situ nitrous oxide production to be of little consequence at any of the three successional stands. Similarly, the acetylene-amended ABS tubes gave little indication that denitrification

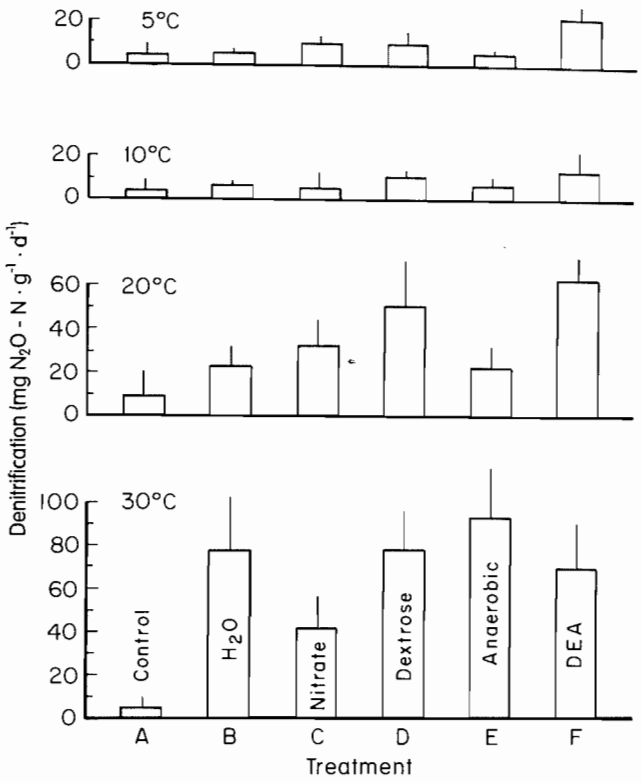

FIG. 2. The effects of temperature (5, 10, 20, and 30°C) and treatment on denitrifying activity in the alder–poplar forest floor. Treatments: A, control (no additions); B, distilled water added to water holding capacity; C, nitrate addition; D, dextrose addition; E, anaerobic atmosphere; F, complete DEA medium addition. Data are presented as means ± 1 SE ($n = 4$).

is a sustained process over time. Rather, highest denitrification activities were observed briefly in the open willow stand after a late summer flood and in laboratory intact cores during the September sampling of poplar–alder forest floor samples. The postflood denitrification in the open willow stand was most likely due to the rapid decomposition of trapped organic matter between the newly deposited alluvium and the old surface mineral soil. The increased poplar–alder forest floor denitrifying activity could be attributed to more available nitrate due to decreasing autumnal plant and (or) microbe competition. The high poplar–alder denitrifying activity measured in September (22 mg N_2O-N·m^{-2}·d^{-1}; using a bulk density conversion factor) was comparable to intermediate rates (30–80 mg N_2O-N·m^{-2}·d^{-1}) measured by Robertson and Tiedje (1984) in midsuccessional temperate hardwood forests, and yet when expressed as a rate per organic matter (OM) (371 ng N_2O-N·g $OM^{-1}h^{-1}$), they are also comparable to the highest rates observed by Melillo et al. (1984) in a 2-year-old hardwood clearcut (560 ng N_2O-N·g OM^{-1}·h^{-1}). The lower denitrifying activities observed throughout the summer at all stages were of the same magnitude as those measured by Ambus and Lowrance (1991) in riparian soils of the Georgian coastal plain.

The laboratory intact core assays, measured using longer incubation periods (24 h at approximately 22°C), had higher denitrifying activities but also had cores with extremely low rates or no detectable activity. The intact core assays also had no observable N_2O production without an acetylene addition,

TABLE 3. The effects of temperature and treatments on denitrifying activity in open willow, poplar–alder, and white spruce mineral soils (MS) and in white spruce forest floors (FF)

Soil	Treatment	Rate of denitrification (ng N_2O-N·g^{-1}·d^{-1}) 5°C	10°C	20°C	30°C
Poplar–alder MS	Control (A)	—	—	—	129±32
	Water (B)	—	—	—	—
	Nitrate (C)	—	—	650±69	3015±110
	Dextrose (D)	—	—	—	736±19
	Anaerobic (E)	—	—	—	—
	DEA (F)	16±3	245±48	1988±108	1243±56
Open willow MS	Control (A)	—	—	—	—
	Water (B)	—	—	11±10	11±5
	Nitrate (C)	—	—	10±1	601±17
	Dextrose (D)	—	—	—	4±4
	Anaerobic (E)	—	—	10±1	10±6
	DEA (F)	—	7±3	358±20	603±22
White spruce MS	Control (A)	—	—	—	—
	Water (B)	—	—	—	—
	Nitrate (C)	—	—	—	—
	Dextrose (D)	—	—	—	—
	Anaerobic (E)	—	—	—	—
	DEA (F)	31±3	—	33±4	33±18
White spruce FF	Control (A)	—	—	—	—
	Water (B)	—	—	—	—
	Nitrate (C)	—	—	—	31±8
	Dextrose (D)	—	—	—	—
	Anaerobic (E)	—	—	—	—
	DEA (F)	12±2	18±11	49±4	92±18

NOTE: Each value is a mean ± 1 SE (n = 4). —, Undetected.

suggesting that N_2 is the major end product of denitrification and that N_2O production by nitrifying bacteria (Yoshida and Alexander 1970; Blackmer et al. 1980; Robertson and Tiedje 1987) is not important in these soils. It should be noted that extremely low rates of both nitrous oxide production and denitrification activity could have been undetectable, as a result of the low sensitivity of the ^3H detector used in N_2O analyses.

The DEA assay measures the concentration of functional denitrifying enzymes in a sample at the time of the assay, due to the addition of the protein-synthesis inhibitor chloramphenicol (Tiedje et al. 1989). We used this assay in two ways: (*i*) to measure DEA on a seasonal scale (monthly), along with intact core activity; (*ii*) to demonstrate limiting factors of denitrification (measured once in September) at each successional stage. The monthly DEA measurements, both in the open willow and in the white spruce mineral soils, showed no seasonal variation, whereas the midsuccessional poplar–alder stand (including forest floor and mineral soil samples) and the white spruce forest floor had increased DEA late in the summer and in early fall. As other researchers have found (Groffman and Tiedje 1989; Ambus and Lowrance 1991), it is difficult to determine short-term relations (daily or monthly) between intact core denitrification and DEA. Instead, long-term annual relationships between DEA and field denitrification have been suggested. Groffman and Tiedje (1989) found linear responses between DEA and the annual denitrification N loss (DEA / N loss ratio) and between DEA and the denitrifying portion of the total microbial biomass (DEA / annual mean DEA to biomass C, or DEA / denitrifying biomass ratio). Their observations indicated that the DEA / denitrifying biomass ratio was a better predictor of annual N loss. This was not evident at our sites. The DEA / N loss ratios of the three mineral soils from the early, middle, and late successional stages were similarly low (7–9) to that of Groffman and Tiedje's (1989), but those of the forest floors of the poplar–alder and white spruce stands were exceptionally high (197 and 476, respectively). The same was determined for the DEA / denitrifying biomass relationship (microbial biomass estimates were measured along with the denitrifying activities and DEA; Klingensmith 1988); the mineral soils were well within the expected ranges, whereas the forest floors had extremely low ratios. In this study, relations of DEAs and biomass suggest strong limiting factors of denitrification in both the poplar–alder and white spruce forest floors, even though denitrifying activity was always greater in the poplar–alder forest floor.

Limitations of denitrification activity were explored by singling out the major controlling factors of denitrification and testing their effects on DEA. This experiment also supported the findings of the in situ and intact core experiments in that denitrifying activity is generally low in the early and late successional stages, whereas higher activities are found in the middle poplar–alder stage. The poplar–alder forest floor had the highest denitrifying activities with increased temperatures and additions of both nitrate and carbon. Increased responses in the poplar–alder forest floor at lower temperatures were observed only with the inclusion of the full DEA media. It was only in the late-successional white spruce stand that temperature had no apparent effect on denitrifying activity, as increases were only observed with the addition of the complete DEA medium regardless of temperature. The suggested

TABLE 4. In situ ABS core tube nonsymbiotic nitrogenase activity in the open willow alluvium and the forest floors (FF) of the poplar–alder and white spruce stands

Sampling date	Rate of nitrogenase activity (μmol $C_2H_4 \cdot m^{-2} \cdot d^{-1}$)			
	Open willow		Poplar–alder FF	White spruce FF
	Fresh alluvium*	New–old†		
July 30	—	710±192*a*	—	351±30*b*
August 15	—	204±25*b*	—	544±38*a*
August 28	148±18*a*	34±11*c*	43±15*a*	38±23*c*
September 9	142±14*a*	—	—	79±5*c*

NOTE: Each value is a mean ± 1 SE (n = 10). Values in a column followed by the same letter are not significantly different ($P < 0.05$). —, Undetected.

*Freshly deposited surface alluvium.

†The interface between the deposited alluvium and the old surface.

TABLE 5. Excised alder root nodule nitrogenase activity

Sampling date	Rate of nitrogenase activity	
	μmol $C_2H_4 \cdot$ g dry wt. nodule$^{-1} \cdot h^{-1}$	mmol $C_2H_4 \cdot m^{-2} \cdot d^{-1}$
July 28	13*	48
August 15	18±8*a*†	66
August 28	5±1*a*†	18
September 9	3±1*a*†	11
September 18	9±3*a*†	33

NOTE: Values followed by the same letter are not significantly different ($P < 0.05$).

*n = 1.

†Mean ± 1 SE (n = 10).

multifactor limitations may explain the basis of the high DEA / N loss ratios of the middle and late successional stage forest floors, but it does not explain the mechanism. Within the floodplain successional sequence, the alder–poplar forest floor has the highest N concentration (2.5%), the lowest C/N ratio (15), and a low lignin/N ratio (14), whereas the white spruce forest floor has a lower N concentration (0.86%), a higher C/N ratio (28), and a higher lignin/N ratio (28) (Van Cleve et al. 1993, this issue). The microbial biomass estimates of the poplar–alder forest floor (20 643 μg C·g soil^{-1}) and the white spruce forest floor (11 234 μg C·g soil^{-1}) are more likely representative of the labile C pool, rather than an accurate estimate of the biomass C (Klingensmith 1988). Limitations of the chloroform fumigation technique to measure biomass C in high organic matter material is known (Vance et al. 1987) and should be used with caution. Yet, together the C/N ratios, the lignin/N ratios, and the biomass C estimates used as an indicator of a labile C pool size suggest that strong C and N competition may cause the lower than expected field denitrifying activity in the poplar–alder forest floor, whereas the low N pool, lower biomass, along with the more recalcitrant organic materials, are dominant factors in controlling denitrification in the later successional, white spruce stand.

Asymbiotic nitrogenase activities are typically reported as <1 kg N·ha^{-1}·year^{-1} (Granhall 1981; Nohrstedt 1984; Grant and Binkley 1987). Our yearly extrapolated rates reflect these low rates, but short-term measurements of acetylene reduction indicate high daily activities in the white spruce moss community and also in association with the flood in the open willow stand. Alexander and Billington (1986) attribute nitrogenase activity in interior Alaskan black spruce (*Picea mariana* (Mill.) B.S.P.) moss to a cyanobacteria–moss association and measured rates of 0.028–27 g N·ha^{-1}·d^{-1} (acetylene reduction to N-fixed conversion factor, 3:1, of Stewart et al. 1967). Using the same conversion factor, our measurements in the white spruce moss community ranged from 7 to 50 g N·ha^{-1}·d^{-1}. Although the laboratory experiments showed no significant response of acetylene reduction to temperature or moisture, this does not necessarily reflect differences that could be due to long-term conditioning. The black spruce stand is typically colder and wetter than floodplain white spruce stands (Viereck et al. 1986), thus the higher observed rates in the white spruce stand could result from a relatively warmer environment. In the open willow stand, immediately after the floodwater receded, the highest measured nonsymbiotic nitrogenase activity of any of the three stands was observed in the new alluvial – old mineral soil interface, with a subsequent decline during each successive sampling period until there was no detectable nitrogenase activity. Anaerobic conditions may have stimulated free-living heterotrophic N fixation (Tiedje et al. 1984) in the trapped organic matter layer. We observed nitrogenase activity in the freshly deposited alluvium approximately 1 month after the flood had occurred. This increase in nitrogenase activity may have been due to the establishment of a free-living cyanobacteria community.

Poplar–alder forest floor nonsymbiotic nitrogenase activities were low to undetectable, but the symbiotic relationship of the actinomycete *Frankia* and alder showed the greatest amount of nitrogenase activity. Van Cleve et al. (1971) calculated the average annual N increment for an alder stand in the same floodplain ecosystem as this study for a 20-year period as 156 kg N·ha^{-1}·year^{-1}. This was estimated by measuring by total soil N and total alder N of differing age-classes. Using the 3:1 conversion factor (Stewart et al. 1967) as a gross approximation of the amount of N fixed, we calculate an annual mean alder nitrogenase activity of 164 kg N·ha^{-1}· year^{-1}. This estimate is most likely conservative, even though it is similar to that of Van Cleve et al. (1971). Giller (1987) and Winship and Tjepkema (1990) point out serious errors that need consideration when using acetylene reduction to estimate N fixation. Included among the many are excised nodules, a closed incubation chamber, long incubation periods, and conversion factors, all of which were used in this study. There are alternative methods that address these sources of error, but none are well adapted for field measurements of mature trees. Even so, the acetylene-reduction technique does give a base-line estimate of nitrogenase activities on a relative scale.

Overall, these results suggest that the estimated alder nitrogenase activity contributes a substantial amount of N to the floodplain ecosystem in general and is a major contributor to the overall higher rates of N processes, such as N mineralization and nitrification, observed in the poplar–alder stand (Klingensmith and Van Cleve 1993, this issue), yet nonsymbiotic N fixation also is a significant contributor in early and late successional stages. Low-terraced, early-successional nonsymbiotic N fixation, associated with flooding, appears to be an episodic contributor of N. Prevegetated stages have little organic C and very low amounts of N; what is found is due to organic materials carried down river and deposited with siltation (Van Cleve et al. 1993, this issue). As vegetation

becomes established on these lower terraced stages, flooding can trap litter and other organic material between deposited silt layers causing a flush of rapid decomposition including anaerobic N fixation and denitrification. Both of these processes were measured after floodwaters had receded from the open willow stage. Here, denitrifying activity was detected only at one sampling time, whereas nitrogenase activity continued for several weeks within the trapped organic layer and also became active on the surface of the newly deposited alluvium. Under these conditions N input from asymbiotic N fixation could be a significant portion of the total N pool. On these early-successional terraces, good drainage and high evaporation rates decrease the waterlogging effect (Van Cleve et al. 1993, this issue), thus decreasing the overall potential for denitrification. In the later successional white spruce stand, moss productivity in white spruce stands has been found to be as much as 24% of the white spruce productivity (Oechel and Van Cleve 1986). This would suggest that the sustained N-fixing activity of the moss communities is a significant contributor of N in later successional, white spruce stands. Although the total nonsymbiotic nitrogenase activity is much lower than that associated with symbiotic N fixation in the alder stand, both the open willow and the white spruce stand activities were greater than the measured brief pulse of denitrification after the flood and throughout the summer in the white spruce stand.

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